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Effects of *Centella asiatica* Administration on NT-3 and CDKN2A Levels in Adult Rat Brains

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: This study aims to determine at the effect of *Centella asiatica* L. (CA) on Neurotrophin-3 (NT-3) brain level and Cyclin-dependent kinase inhibitor 2A (CDKN2A) brain level of rats aged 12, 24 and 36 weeks.
Study Design: Experimental studies used rats aged 12, 24, and 36 months which were treated with CA extract 300 mg/BW was administered orally for 29 days in a row as described in previous study. In addition, untreated rats aged 12, 24, and 36 months were used as negative controls.
Place and Duration of Study: Department of Biochemistry and Biology Molecular, Faculty of Medicine, Universitas Indonesia.
Methodology: NT-3 and CDKN2A brain levels were measured using the ELISA method.
Results: The results showed a significant decrease in NT-3 levels in rats aged 36 weeks that were given CA compared to control rats (Sidak multiple comparison test*; P = .0493*). In addition, the CDKN2A levels of CA-treated rats aged 36 weeks that were compared to control rats (Sidak multiple comparison test, *P* = .0041).
Conclusion: This study proved that giving CA 300mg/kgBW for 29 days in adult rats aged 36 weeks has not been able to prevent aging in terms of NT-3 and CDKN2A proteins.

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Keywords: NT-3; CDKN2A; protein; rat brain aged 12, 24 and 36 weeks.

1. INTRODUCTION

The report from the Data and Information Center of the Ministry of Health (Infodatin 2014) regarding the situation and Analysis of the Elderly shows that the life expectancy of the Indonesian population in 2015-2020 is 71.7 years [1]. If the life expectancy of the elderly increases but their health status is not good, it will lead to an increase in the number of state dependents. This can be reduced if we succeed in improving the quality of life for the elderly.

One of the causes of a decrease in the quality of life in elderly people is the degeneration of brain cells (neurodegenerative). In adult humans, the proliferation of nerve cells is very limited. This causes damage to nerve cells in the brain which is medically more dangerous than damage to cells in other organs. Nerve cells that experience cell death (apoptosis) are irreplaceable [2]. Aging itself is a collection of phenotypes characterized by decreased repair and or regeneration of dead or damaged cells [3]. Therefore, efforts are needed so that the quality of life of the elderly is well maintained and avoid various degenerative diseases.

Several plants that pharmacologically show antiaging effects related to antioxidants, which can act as neuroprotective/neurotherapy or reduce some of these degenerative diseases include CA [4–6].

Indonesia has a lot of native plants which are considered by the community to have different properties in treating or preventing a disease. Giving CA and/or *Acalypha indica* (AI) related to its effect as a neuroprotective has been carried out by several researchers [7-9]. In addition, a combination of CA and AI has also been studied which shows an antioxidant effect in hypoxic rats [10,11]. The main chemical components of CA that play a role in its pharmacological activity are triterpenes, mostly asiaticoside, asiatic acid, madecassoside, and madecassic acid. Ethanol, methanol, and water extracts of CA increase nervous system function [12]. In a previous study, administration of CA extract to rats aged 19 months at a dose of 300 mg/kgBW for 29 consecutive days was able to reduce brain carbonyl levels [13]. Based on the results of this study, further research was carried out by giving CA extract with the same dose in adult rats, namely: 12, 24, and 36 weeks. NT-3 protein was measured as a neuroprotective biomarker and CDKN2A was measured as a senescence biomarker.

2. MATERIALS AND METHODS

Thirty male Wistar rats were divided into six treatment groups. 12WCA(-): rats aged 12 weeks that were not given anything; 12WCA(+): rats aged 12 weeks given CA extract 300 mg/kg body weight; 24WCA(-): rats aged 24 weeks that were not given anything; 24WCA(+): rats aged 24 weeks given CA extract 300 mg/kg body weight; 36WCA(-): rats aged 36 weeks that were not given anything; 36WCA(+): rats aged 36 weeks given CA extract 300 mg/kg body weight. CA extract was administered orally for 29 days in a row as described in previous study. After being given treatment, the rats were decapitated and necropsy was performed to take their brain tissue.

2.1 Sample Preparation

Homogenate of rat brain tissue was prepared in 0.01 M Phosphate Buffer Saline (PBS buffer), pH 7.4. The brain homogenate was stored at -80°C until it was time to measure NT-3 and CDKN2A (no more than 3 months) using the ELISA method.

2.2 Measurement of NT-3 and CDKN2A with ELISA Method

The assay was conducted in accordance with the manual guide (Elabscience® and MyBiosource®).The blanks, standards, and samples were loaded into the NT-3 or CDKN2A antibody-coated wells. Then added avidin which is conjugated with the enzyme horseradish peroxidase (HRP). After that, the addition of tetramethylbenzidine (TMB) substrate was carried out. Wells containing the corresponding NT-3 or CDKN2A proteins will show a color change. The enzyme-substrate reaction was terminated by adding sulfuric acid as the stopping solution. The color change was measured using an ELISA reader (Varioskan Flash) at a wavelength of 450 nm. Concentration of the NT-3 or CDKN2A was determined by comparing the optical density of the sample with the standard curve.

2.3 Preparation of *Centella asiatica* (CA) Extract

Extract of CA was prepared from simplicia of dried CA leaves was obtained from PT. Biofarindo makes ethanol extract in the Department of Chemistry, Faculty of Medicine, University of Indonesia [14].

2.4 Statistical Analysis

The results of measuring the levels of NT-3 and CDKN2A were analyzed for data distribution using the Shapiro-Wilk test. Furthermore, comparisons were made between the age groups that were not given CA and those who were given CA. If the distribution of the data is normal, use the two-way ANOVA test than continued with Sidak multiple comparison test.

3. RESULTS AND DISCUSSION

3.1 Brain NT-3 Level

The results of the NT-3 protein ELISA measurements were as follows, 12WCA (-): (0.440±0.883)ng/mL; 12WCA(+): (0.492±0.066) ng/mL; 24WCA(-): (0.393±0.055)ng/mL; 24WCA(+): (0.419±0.056) ng/mL; 36WCA(-): (0.391±0.079) ng/mL; 36WCA(+): (0.285±0.058) ng/mL. The results of statistical analysis showed that the NT-3 protein data was normally distributed. The interaction between the effects of centella and age on NT-3 level was not significant statistically (two-way ANOVA; P = .4544). Simple main effects analysis showed that NT-3 levels of CA (+) group were significantly lower than CA (-) group at 36-week of age (Sidak multiple comparison test; P = .0493), but the differences were not significant between CA (+) and CA(-) groups at 12-week of age (P = .9571) or 24-week of age (P = .4093) (Fig. 1).

NT-3 is a protein belonging to the group of Neurotrophic Factors (NTFs) expressed by neurons and astrocytes of neural networks. NTFs contribute to the regulation of nerve cell progression and regressivity, which determines whether nerve cells will be kept alive or not. NT-3 can protect nerve cells, regulate nerve cell proliferation, stimulate axon and dendrite growth and the formation of myelin sheaths by activating Schwan cells [15]. NT-3 has TrkC, TrkA, and TrkB receptors, all of which mediate almost all neuronal pathways in the central and peripheral nervous system for survival and differentiation [16]. Various other studies have also explained the role of neurotrophins in maintaining neuron cells, proliferation, maturation during the stages of brain development, and neuroprotective function in the adult brain even with the damage [17].

A significant reduction in NT-3 in the 36-week-old rat group that was given CA could imply that CA administration at this age had not yet had a neuroprotective effect on damage to the brain. The Free Radical Theory of Aging states that aging is caused by the accumulation of free radicals [18]. Along with increasing age there is an increase in lipid peroxidation. Research by Zhu Y et al. showed higher results of lipid peroxidation in the striatum, mesencephalon, and cerebellum in older rat brains [19].

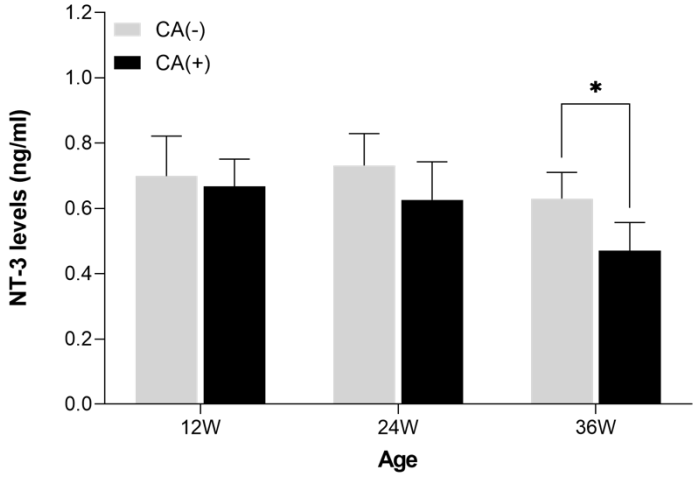

Fig. 1. Comparison level of NT-3 between CA (+) group and CA (-) group
*Sidak multiple comparison test: significant from normal control at 36 weeks, * P < 0.05*

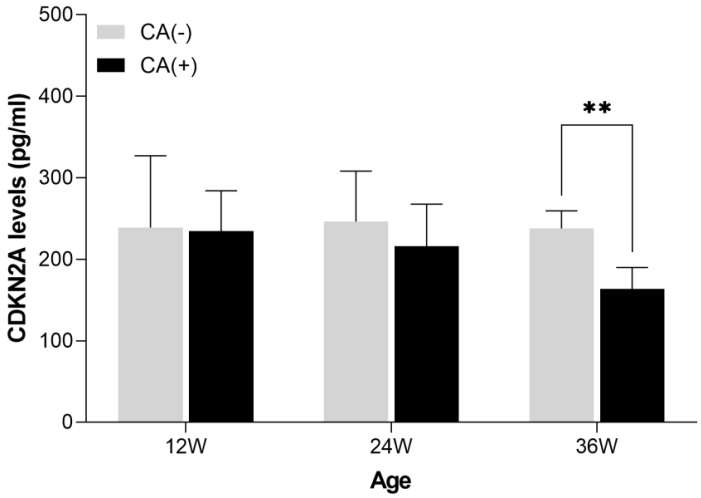

Fig. 2. Comparison level of CDKN2A between CA (+) group and CA (-) group
*Sidak multiple comparison test: significant from normal control at 36 weeks, * P < 0.05*

3.2 Brain CDKN2A Level

The results of measurements of CDKN-2A levels in rat brains were as follows:

12WCA (-): (0.450±0.123)pg/mL
12WCA (+): (0.444±0.069)pg/mL
24WCA (-): (0.460±0.087)pg/mL
24WCA (+): (0.418±0.072)pg/mL
36WCA (-): (0.449±0.030)pg/mL
36WCA (+): (0.345±0.037)pg/mL

The interaction between the effects of centella and age on CDKN2A level was not significant statistically (two-way ANOVA, P = .4185). Simple main effects analysis showed that CDKN2A levels of CA(+) group were significantly lower than CA(-) group at 36-week of age (Sidak multiple comparison test, P = .0041), but the differences were not significant between CA (+) and CA(-) groups at 12-week of age (Sidak multiple comparison test, P = .999) or 24-week of age (Sidak multiple comparison test, P = .999) (Fig. 2).

The tumor suppressor protein $p16^{INK4a}$ (CDKN2A, p16) is a member of the INK4 family of cyclin-dependent kinase inhibitors. This protein plays an important role in cell cycle regulation. Expression of p16 prevents cell proliferation by binding to and inhibiting cyclin-dependent kinases 4 and 6 (CDK4/6) [20]. The activating CDKN2A locus, resulting in $p16^{INK4a}$ and ARF, is found in most senescent cells and may play a causal role in their growth inhibition. The CDKN2A locus is suppressed in normal tissue, becoming activated during tissue damage or cellular stress. Therefore, in healthy young organisms, expression of $p16^{INK4a}$ is low or undetectable, but its expression increases exponentially in most tissues with aging [3,21]. Activation of the $p16^{INK4a}$ promoter was selected as the biomarker of choice for in vivo studies of aging due to its extreme dynamic range and strong association with aging [22–25].

This study measured CDKN2A expression at the protein level, not the gene level. The results showed a significant decrease in CDKN2A protein levels between the group of rats aged 36 weeks that were not given CA compared to those that were given CA (Sidak multiple comparison test, P = .0041). However, in the group of rats aged 12 and 24 weeks, there was no significant difference between those not given CA and those who were given CA. In healthy young organisms, $p16^{INK4a}$ expression is low or undetectable. Giving CA to the group of rats aged 36 weeks decreased significantly, but was not able to completely suppress CDKN2A activation [3,20]. This is also supported by the results of a significant decrease in NT-3 protein levels, which can be interpreted as a decrease in neuroprotective function resulting in cellular damage. Cellular damage may also be caused by higher levels of lipid peroxidation in the striatum, mesencephalon, and cerebellum in older rats [18].

The limitation of this study was not to measure CDKN2A expression at the gene level, but to measure it at the protein level as a senescence

biomarker. In addition, it also did not measure the expression of NT-3 receptor proteins, namely TrkC, TrkA.

4. CONCLUSION

This study proved that giving CA 300mg/kgBW for 29 days in adult rats aged 36 weeks has not been able to prevent aging in terms of NT-3 and CDKN2A proteins. Administration of CA 300mg/kgBW for 29 days to adult rats aged 36 weeks has not been able to increase the neuroprotective role, and still caused the tissue damage or cellular stress.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This research has obtained ethical permission from the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia with Number KET-503/UN2.F1/ETIK/ PPM.00.02/2021.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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